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In vitro Evaluation of the Essential Oil Extract of Six Plant Species and Ivermectin on the Microfilaria Larva of *Simulium yahense*

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ABSTRACT

A comparative laboratory bioassay of the essential oil extracts of *M. koenigii* (seed), *Citrus paradisi* (seed) and the leaves, stem barks and roots of *Alstonia boonei, Alternanthera repens, Eclipta prostrata* and *Rothmannia longiflora* and their combination were compared with ivermectin against the third infectious microfilaria larva of *Simulium yahense*. Bioassay test showed remarkable larvicidal properties for ivermectin and the plant species under study as they could induce significant mortalities at low and varying concentrations in the larva of *S. yahense*. The LC_{50} and LC_{90} values estimated for *M. koenigii, Citrus paradisi, Alstonia boonei, Alternanthera repens, Eclipta prostrata, Rothmannia longiflora*, their combination and ivermectin are 5.75 and 13.18, 8.38 and 15.49, 3.02 and 15.49, 2.472 and 9.33, 2.87 and 12.88, 2.93 and 13.49, 2.61 and 3.16 and 2.43 and 5.37 ppm, respectively. The study revealed that ivermectin and the combination of the plant extracts could induce 100% mortality of the larva at a dose dependent response.

Key words: Combination, essential oil extracts, ivermectin, onchocerciasis, *Simulium yahense*

INTRODUCTION

Onchocerciasis is an infestation with filarial worms of the genus *Onchocerca*, common in tropical America and Africa, transmitted by black flies and characterized by nodules under the skin, an itchy rash, eye lesions and in severe cases, elephantiasis. The principal clinical manifestations are ocular lesions, resulting in visual, impairment and blindness and Onchocercal Skin Disease (OSD) (Murray and Lopez, 1996). Szirmai (2005) estimated that West Africa share about 30% of global cases of onchocerciasis with 96% of reported cases globally occurring in Africa. More so, this disease burden has contributed significantly to increased incidence of eye defects in tropical West Africa. For example Dadzie *et al*. (1989) reported an onchocerciasis prevalence ratio of 1:3 to other ocular problems in North central and North eastern part of Nigeria. Like most other diseases, majority of infestation cases are hardly reported nor documented. Over the years, control and management of the larvicidal activities of this epidemic has been achieved with diethyl carbamazine and most notably with ivermectin. Ivermectin marketed under the brand name Stromectol is administered orally and available in 3 mg tablets containing microcrystalline cellulose, pregelatinized starch, magnesium separate, butylated hydroxyanisole and citric acid powder (anhydrous). It is derived from the avermectins, a class of highly active broad-spectrum, anti-parasitic agents isolated from

the fermentation products of *Streptomyces avermitilis*. It is a white to yellowish-white, nonhygroscopic, crystalline powder with a melting point of about 155°C. It is insoluble in water but is freely soluble in methanol and soluble in 95% ethanol. The required dosages for patients between ages 15-25, 26-44, 46-64, 65-84 and above 84 are one, two, three, four and 150 mcg kg^{-1} of 3 mg tablets, respectively. However, clinical studies have shown that stromectol (ivermectin) has no activity against adult *Onchocerca volvulus* parasites since they reside in subcutaneous nodules which are infrequently palpable. Surgical excision of these nodules (nodulectomy) is often considered in the management of patients with onchocerciasis, since this procedure will eliminate the microfilaria-producing adult parasites (Ali *et al*., 2003). More so, the use of this drug is fraught with vision changes, urinary or bowel problems, weakness, confusion, lack of coordination, eye redness, swelling, pain or seizure (convulsions) (De Sole *et al*., 1989, 1991). It is in view of these shortcomings, that researches using cheap, readily available and negligible side effects from natural plant products are being championed by the Federal Ministry of Health in Nigeria. At the moment, priority is being paid to the application of these natural plant products through the use of larvicides and ovicides as ideal control measures. Natural products and secondary metabolites formed by living systems, notably from plant origin, have shown great potentials in treating human diseases such as cancer, coronary heart diseases, diabetes, malaria and infectious diseases. It is against this back drop that this study aims at evaluating the larvicidal properties of these six plant species, their combination and ivermectin against the infectious larva stage of *S. yahense*. In the present investigation, specific action of each plant, their combination and ivermectin were evaluated against the third infectious stage (microfilaria) of *S. yahense*.

MATERIALS AND METHODS

Sample collection: Six medicinal plants were collected from Ugbodu in Aniocha North local government area of Delta state, Nigeria. Seven herbalists from randomly selected communities in the area were interviewed on their methods of treatment of onchocerciasis which is prevalent in the area. After the interview six plants *M. koenigii* (seed), *Citrus paradisi* (seed) and the leaves, stem barks and roots of *Alstonia boonei, Alternanthera repens, Eclipta prostrata* and *Rothmannia longiflora* were mentioned as anti-onchocerciasis herbs and two seeds were mentioned as possessing anti larvicidal properties. All of them were unanimous in their use of the herbs in combination for enhanced efficacy and their mode of preparation of the herbs (boiling or maceration in alcohol). The seeds of the plants were obtained through the help of two herbalists from the village forest. In addition, their respective leaves and stems together with those of the other mentioned plant species were collected for botanical identification. The identification and authentication was done at the herbarium unit of the Department of Botany, University of Calabar, Nigeria, where a voucher specimen was deposited.

Methods: The freshly collected plant specimens were washed thoroughly, weighed and chopped off finely and subjected to hydro-distillation in a Clevenger's apparatus in order to obtain its vaporizing essential oils. The oil color ranged from yellow through red to dark brown and black, with a moderate to low viscosity and a yield of 2.438, 2,745, 2.314, 4.311 3.845, 3.362 and 6.497 mL kg^{-1} weight of the starting material. The oil was then dissolved in acetone in a ratio of 1:1. A series of concentrations ranging from 3-15 ppm of the dissolved oil were prepared in distilled water. Similarly, 3 mg tablets of ivermectin were grounded to powder and used to prepare similar concentrations to that of the plants essential oil. Three replicates were run for each concentration

and the plant extract/ivermectin. The oil obtained was stored in screw capped vials at laboratory conditions until tested. Control tests were carried out in parallel with the required amount of acetone in water. The extraction was carried out n the department of Biochemistry, UNICAL.

Supply of ivermectin: Ivermectin was obtained as a classified drug with No. 8495 and supplied as NDC 0006-0032-20, with unit dose packages of 20, storage temperature below 30°C (86°F) and an expiring shelf life of 06-09-2012. STROMECTOL (ivermectin) 3 mg are white, round, flat, bevel-edged tablets coded MSD on one side and 32 on the other side, distributed by MERCK and CO., INC, Whitehouse Station, NJ 08889, USA and manufactured by MSD BV Waarderweg 39 2031 BN Haarlem Netherlands.

Larva collection and laboratory bioassay: The microfilaria larvae were prepared and obtained from Nigeria Institute for pharmaceutical research and development, Idu, Abuja. Pure culture of the larva containing twenty-five microfilaria each were prepared and stored in glass vials at 27±0.2°C. The larvae were provided a mixture of Dog biscuit, yeast powder and algae in a ratio 3:1:1 ratio. Twenty-five larvae were released into 500 mL glass beakers and supplemented with treatments by taking 3.0, 6.0, 9.0, 12.0 and 15.0 ppm of plants essential oil and ivermectin, respectively. Mortally counts were made after 24 h exposure. Bioassay test showing more than 15% control mortality was discarded and repeated. However, when control mortality ranged from 5-15%, the corrected mortality was corrected using Schneider-Orelli's formula (Puntener, 1981). The data obtained were subjected to probit analysis in order to estimate the LC_{50} and the heterogeneity values (Finney, 1952). The experiment was carried out at 27±0.2°C.

RESULTS AND DISCUSSION

Nowadays, the trend in the control and management of infectious diseases is directed against larval and only against adult when necessary (McConnell *et al*., 2010). This is because the fight against adult is temporary, unsatisfactory and polluting for the environment, while larval treatment is more localized in time and space resulting in less dangerous outcomes. Larval control and the use of natural products can be an effective tool in onchocerciasis management since clinical studies has shown that Stromectol (ivermectin) has no activity against the adult stage (Burnham, 1995). In the present study, the test revealed that at concentrations of 9, 12 and 15 ppm of the essential oil extract combination, *Alternanthera repens* and ivermectin, could induce 100% larval mortality. The larvicidal responses of the two remedies were shown to be dose dependent. The data obtained was subjected to probit analysis in order to estimate the LC_{50} and LC_{90} and heterogeneity values. The LC_{50} and LC_{90} values estimated for *M. koenigii*, *Citrus paradisi, Alstonia boonei, Alternanthera repens, Eclipta prostrate, Rothmannia longiflora*, their combination and ivermectin are 5.75 and 13.18, 8.32 and 15.49, 3.02 and 15.49, 2.61 and 9.33, 2.87 and 12.88, 2.93 and 13.49, 2.47 and 5.46 and 2.43 and 5.37 ppm. The essential oil of the combination remedy was found to be relatively more toxic to the microfilaria larva (1:1), followed by that of *Alternanthera repens* (2:1) while the activity of *Murruya koenigii* oil extract was shown to be relatively least active (3:1) when compared to ivermectin.

The calculated chi-square values for ivermectin, combination, *Rothmannia longiflora* and *Alternanthera repens* are less than table values at 0.05% (Table 1) indicating random variations among the doses for the aforementioned remedies.

Table 1: Dosage response of the essential oil of six plant species, their combination and ivermectin against the microfilaria larvae of *Simulium yahense*

Extract	LC_{50} values (ppm)	LC_{90} values (ppm)	Heterogeneity values-χ^2 test (df *)	Relative toxicity
Citrus paradisi	5.75	13.18	13.88 (3)	2:1
Murraya koenigii	8.32	15.49	39.63 (3)	3:1
Alstonia boonei	3.02	15.49	36.80 (3)	2:1
Alternanthera repens	2.61	9.33	7.12 (3)	1:1
Eclipta prostrata	2.87	12.88	26.01 (3)	2:1
Rothmannia longiflora	2.93	13.49	5.79 (3)	2:1
Combination	2.47	5.46	2.25 (3)	2:1
Ivermectin	2.43	5.37	2.23 (3)	

Ppm: parts per million; LC_{50}: Lethal concentration needed to kill 50% of larva exposed; LC_{90}: Lethal concentration needed to kill 90% of larva exposed; *df: Degree of freedom; χ^2: Chi-square for heterogeneity

Previous literatures on larvicidal control of microfilaria have been carried out through spraying of endemic breeding sites over a long-distant range (Remme, 2004). Other control measures include, epidemiological modeling (Remme *et al*., 1995), research on disease patterns and community trials of ivermectin (WHO, 1995). Such control measures are either subjective, environmentally hostile or fail to achieve target objective. There have been dearths of published works on the use of natural products of plant origin in the larvicidal control of onchocerciasis.

CONCLUSIONS

This study indicates that the essential oil extract of *M. koenigii, Citrus paradisi* and the the leaves, stem barks and roots of *Alstonia boonei, Alternanthera repens, Eclipta prostrata* and *Rothmannia longiflora* and their combination and ivermectin has remarkable larvicidal activity against *Simulium yahense*. It is noteworthy to study extensively the larvicidal properties of the essential oils contained in the plants by isolating and identifying the active ingredients that cause larval mortality and then use them in field trials in order to assess their potentials as alternative to ivermectin and other chemical larvicides whose activities are being reviewed.

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